PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	ISHII *et al.*	EXAMINER:	POPA, ILEANA
APPLICATION NO.:	10/680,356	ART UNIT:	1633
FILED:	OCTOBER 6, 2003	CONF. NO:	5651

FOR: **SPATIALLY ENCODED AND MOBILE ARRAYS OF TETHERED LIPIDS**

APPELLANTS' BRIEF ON APPEAL

Mail Stop: Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
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Sir:

This is an appeal to the Board of Appeals and Interferences from the Final Office action of Examiner Popa mailed January 5, 2011 (hereafter "Final Office action") in which pending claims 1-4, 6-12 and 22-25 stand in final rejection.

STATEMENT OF THE REAL PARTY IN INTEREST

The real party in interest is The Board of Trustees of the Leland Stanford Junior University, assignee of record.

RELATED APPEALS AND INTERFERENCES

Appellants are not aware of other appeals or interferences which would directly affect or be directly affected by or have a bearing on the Board's decision in the present appeal.

STATUS OF CLAIMS

Claims 1-4, 6-12, and 22-25 are pending and are the subject of this appeal. Claims 13-18 and 21 have been withdrawn. Claims 5 and 19-20 have been cancelled. The pending and withdrawn claims are presented in the Claims Appendix.

STATUS OF AMENDMENTS

No amendment was filed subsequent to the final rejection of the claims in the Final Office action.

SUMMARY OF CLAIMED SUBJECT MATTER

The sole independent claim 1 is directed to an array of separated lipid bilayers (page 1, lines 9-10). The array comprises a substrate (page 13, lines 7-11) having a surface defining a plurality of distinct bilayer-compatible surface regions (page 13, lines 12-13); a plurality of discrete lipid bilayer expanses in associated surface regions, the expanses having inner and outer bilayer surfaces (page 13, lines 12-14 and 18); an aqueous film interposed between each bilayer-compatible surface region and the lower surface of the corresponding lipid bilayer expanse (page 13, lines 19-21); a bulk aqueous phase covering the lipid bilayer expanses (page 13, lines 23-24); and at least one biomolecule anchored to at least one of the lipid bilayer expanses through a complementary oligonucleotide sequence capable of specifically hybridizing with the surface region specific oligonucleotide sequence in that expanse (page 14, lines 16-21), such that the biomolecule is anchored to that expanse. One or more of the biomolecules is a vesicle (page 10, line 29 to page 11, line 7 and page 14, lines 21-22) including at least one receptor associated with the vesicle (page 10, lines 25-29). The receptor has a binding site located on the exterior of the vesicle and being capable of specifically binding a test agent (page 11, lines 25-29 and Fig. 10). Each of the expanses containing one or more lipids derivatized with an oligonucleotide has a surface region specific oligonucleotide sequence and extending from the outer surface of the associated expanse (page 14, lines 9-12).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There are three issues for review on appeal.

1. The first issue is whether claims 1-4, 6-7, 9, 11-12, and 22-25 are obvious under 35 U.S.C. § 103 by Boxer *et al.* (PCT Publication No. WO 98/23948) in view of both Boukobza *et al.* (J. Phys. Chem, 2001, 105:12165-12170) and Niemeyer *et al.* (German Publication No. 19902391).

2. The second issue is whether claims 1-4, 6-7, 9-12 and 22-25 are obvious under 35 U.S.C. §103 over Boxer *et al.* taken with both Boukobza *et al.* and Niemeyer *et al.* in further view of each of Cornell *et al.* (U.S. Patent No. 5,874,316) Arnold *et al.* (U.S. Patent No. 5,310,648) and Bayerl *et al.* (U.S. Patent No. 6,051,372).

3. The third issue is whether claims 1-4, 6-9, 11-12 and 22-25 are obvious under 35 U.S.C. §103 over Boxer *et al.* taken with both Boukobza *et al.* and Niemeyer *et al.* in further view of Shen *et al.* (U.S. Publication No. 2003/0148335).

ARGUMENT

I. Rejection of claims 1-4, 6-7, 9, 11-12, and 22-25 over Boxer *et al.* taken with both Boukobza *et al.* and Niemeyer *et al.*

Summary of Cited References

BOXER ET AL. relate to a surface detector array formed of a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions. The bilayer-compatible surface regions may further include a selected biomolecule covalently or non-covalently attached to a lipid molecule (see page 4 line 32 through page 5, line 2). Examples of biomolecules include polynucleotides and nucleic acids (see page 5, lines 4-5 and page 16, line 4). The bilayer may be derivatized with groups or compounds to create a surface having the desired surface exemplified by a ligand bound to the surface of the lipid by attachment to surface lipid components (see page 11, line 32 through page 12, line 2). Specific high-affinity molecular interactions may be employed to link biomolecules to a supported layer (see page 18, lines 7-8).

BOUKOBZA ET AL. describe an immobilization technique using biotin-avidin interaction. Large unilamellar lipid vesicles (LUV) are attached to a glass-supported lipid

bilayer through the biotin-avidin binding interaction. The LUV includes a single biomolecule encapsulated inside the LUV for characterization of the biomolecule.

NIEMEYER ET AL. states reversible, parallel, site-specific immobilization of macromolecules on a solid phase comprising using nucleic acids as immobilization-mediating reagents. The components to be immobilized on the solid support are coupled with nucleic acids and the solid phase are functionalized with nucleic acids complementary thereto.

The Combination of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* Fail to Teach All the Claimed Elements

The Board of Patent Appeal and Interferences has confirmed that a proper, post-KSR obviousness determination requires the Office make "a searching comparison of the claimed invention – including all its limitations – with the teaching of the prior art." *Ex parte Wada and Murphy*, Appeal 2007-3733 (2008), citing *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) and *CFMT v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003). "It is well settled that the 'Patent and Trademark Office (PTO) must consider all claim limitations when determining patentability of an invention over the prior art.'" *Ex parte Wada and Murphy*, Appeal 2007-3733 (2008), citing *In re Lowry*, 32 F.3d 1579, 1582 (Fed. Cir. 1994).

The combination of Boxer *et al.*, Boukobza *et al.*, and Niemeyer *et al.* cannot render the present claims obvious as the combination fails to teach the claimed array as a whole. Specifically, the combination fails to show or suggest an array of separated lipid bilayer expanses including at least one vesicle including a receptor associated therewith, the vesicle anchored to a lipid bilayer expanse through complementary oligonucleotides.

As acknowledged by the Examiner, Boxer *et al.* does not teach vesicles at all much less receptors associated with the vesicle and having a binding site located on the exterior of the vesicle (Final Office action page 4, lines 3-6). The Examiner points to Boukobza *et al.* for teaching an immobilization technique for trapping single protein molecules within a lipid vesicle tethered to a lipid bilayer by biotin-avidin interactions for fluorescence studies (Final Office Action page 4, lines 7-10). The Examiner asserts

that it "would have been obvious to one of skill in the art to associate the receptors with the vesicles to achieve the predictable result of obtaining a device suitable to detect the interactions between the receptors and their ligands" (Final Office action page 4 line 20 to page 5, line2). Further, the Examiner cites Niemeyer *et al.* for tethering via oligonucleotide hybridization. Appellants submit that the Examiner's reasoning is legally and technically wrong, and that the cited references do not render obvious the claimed device.

A challenge to using lipid bilayer arrays on a solid substrate for large proteins such as receptors is that the protein may extend beyond the lipid bilayer and interact with or drag on the substrate. This interaction can cause denaturation and loss of function of the protein or limit the lateral mobility of the lipid bilayer on the substrate. By having the vesicle tethered to the bilayer expanse through complementary oligonucleotides as in the present claims, the vesicle and associated receptor are distanced from the substrate. Therefore, the presently claimed array retains the benefits of spatial organization and lateral mobility from the lipid bilayer on a substrate while retaining the function of the receptor.

Boxer *et al.* describe a lipid bilayer array with a biomolecule attached to a lipid of the lipid bilayer but make no mention of the biomolecule being a vesicle (Abstract). Boukobza *et al.* teach using biotin-avidin affinity for tethering a lipid vesicle having an entrapped biomolecule to a surface supported lipid bilayer. As seen in Figure 1 on page 12166, Boukobza *et al.* describes a liposome that includes a protein encapsulated within the liposome. However, the liposome of Boukobza *et al.* does not teach or suggest a vesicle including at least one receptor associated with the vesicle and having a binding site located on the exterior of the vesicle and being capable of specifically binding a test agent. Instead, the liposome is used to confine a single biomolecule within the liposome for fluorescence studies of the biomolecule. Nor would one modify Boukobza *et al.* to include a receptor having a binding site located on the exterior of the vesicle as Boukobza *et al.* is concerned with immobilization of the biomolecule so that it can be studied by single-molecule fluorescence. Such a procedure uses long measurement times and thus the molecule must be immobilized. Entrapment within the liposome allows the molecule to be immobilized within the liposome but retain freedom

of motion for fluorescence polarization (see Abstract). Niemeyer *et al.* teaches immobilization of a biomolecule, which may be a vesicle, to a solid support through complementary oligonucleotide sequences. However, Niemeyer *et al.* makes no mention of including a receptor associated with the vesicle having a binding site located on the exterior of the vesicle and being capable of specifically binding a test agent. Therefore, this reference combined with Boxer *et al.* and/or with Boukobza *et al.* does not show or suggest all the features of claim 1.

At most, the combination of references taken as a whole can be said to teach a bilayer array as in Boxer *et al.* with a vesicle immobilized to the bilayer array through complementary nucleic acids as in Niemeyer *et al.* and Boukobza *et al.*, in part. Nowhere does the combination show or suggest anchoring a vesicle having at least one receptor *having a binding site located on the exterior of the vesicle* and being capable of specifically binding a test agent above the lipid bilayer using complementary oligonucleotides so that the vesicle and/or receptor does not interact with the substrate.

The Combination of Boxer *et al.* in view of Boukobza *et al.* and Niemeyer *et al.* Fails to Provide an Articulated Reasoning to Support the Legal Conclusion of Obviousness

Even after *KSR*, it remains necessary to show "some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness". *KSR* at 418, citing *In re Kahn*, 441 F.3d 977, 988 (C.A. Fed. 2006). Further, the Office examination guidelines following the Court decision in *KSR* describe six rationales that many be used to support a rejection under 35 U.S.C. § 103. See Federal Register, Vol. 72, No. 195, October 10, 2007.

In making the present rejection, the Examiner appears to be relying on Rationale A - Combining Prior Art Elements According to Known Methods to Yield Predictable Results to support the rejection. A rejection based on this rationale must articulate: (1) a finding that the prior art included each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference; (2) a finding that one of ordinary skill in the art could have combined the

elements as claimed by known methods, and that in combination each element merely would have performed the same function as it did separately; (3) a finding that one of ordinary skill in the art would have recognized that the results of the combination were predictable; and (4) whatever additional findings based on the Graham factual inquiries may be necessary...to explain a conclusion of obviousness.

As noted above, the Examiner fails articulate a finding that the prior art included each element claimed.

One Skilled in the Art Would Not Modify Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* to Arrive at the Presently Claimed Array

The Examiner has provided no reasoning for one skilled in the art to modify the combined teaching of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* along an untaught and unsuggested path to arrive at the presently claimed array. The Examiner states, with regard to Boukobza *et al.*, the technique "overcomes the problem of molecule-surface interaction and wherein the surface-tethered vesicles can be used for experiments on reconstituted membrane proteins and peptides (i.e. a vesicle capable of specifically binding a test agent)" (Final Office action page 4, lines 11-13). While Boukobza *et al.* does teach immobilization of a vesicle to "overcome the problem of molecule-surface interaction" (page 12165, second column), Boukobza *et al.* makes no mention of binding a vesicle to a test agent. Instead, the protein molecule is trapped *inside* the vesicle to immobilize the molecule for fluorescence studies. The Examiner states that "it would have been obvious to one of skill in the art to associate the receptors with the vesicles to achieve the predictable result of obtaining a device suitable to detect the interactions between the receptors and their ligands" (Final Office action, paragraph bridging pages 4 and 5) without giving any reasoning for such an assertion. Niemeyer *et al.* teach the use of nucleic acids as immobilization mediating reagents to immobilize a biomolecule to a support "in order to be able to use cost-intensive sensor surfaces several times" (page 2, lines 17-18) and to immobilize "many different macromolecules in a single reaction step (page 3, lines 10-11). A lipid bilayer would not be reusable and the ability to immobilize many different macromolecules in one step does not suggest to one skilled in the art to modify the teaching along the lines

of the present array. Thus, the Examiner has not articulated a reason for the untaught and unsuggested modification of the presently cited references along the lines of the present claimed array.

II. Rejection of claims 1-4, 6-7, 9-12 and 22-25 over Boxer *et al.* taken with Boukobza *et al.* and Niemeyer et all, in further view of Cornell *et al.*, Arnold *et al.*, or Bayerl *et al.*

The Examiner cites Cornell *et al.*, Arnold *et al.*, and Bayerl *et al.* for the sole purpose of providing features of dependent claim 10 - self-limiting lateral diffusion. Thus, for the prima facie case of obviousness to stand, the combined teachings of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* must provide all of the features of independent claim 1. For the reasons given above, it is abundantly clear that the combined teachings of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* do not provide all of the features of claim 1. Nor do any of Cornell *et al.*, Arnold *et al.* or Bayerl *et al.* provide the missing teaching. Cornell *et al.* relate to receptor binding of an analyte. As seen in Fig. 1A, receptor molecules (16) are attached to an end of ionophores (15) incorporated in a membrane (10). Arnold *et al.* describes an imprinted matrix which exhibits selective binding interactions through metal chelates (Fig. 3B). Bayerl *et al.* describe two-dimensional patterning of a three-dimensional surface by a template molecule. Therefore, a prima facie case of obviousness of claim 10 has not been established.

III. Rejection of claims 1-4, 6-9, 11-12 and 22-25 over Boxer *et al.* taken with Boukobza *et al.* and Niemeyer *et al.*, in further view of Shen *et al.*

The Examiner cites Shen *et al.* for the sole purpose of providing features of dependent claim 8 - the identity of a biomolecule being determined from the sequence of the oligonucleotide. Thus, for the prima facie case of obviousness to stand, the combined teachings of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* must provide all of the features of independent claim 1. For the reasons given above, it is abundantly clear that the combined teachings of Boxer *et al.*, Boukobza *et al.* and Niemeyer *et al.* do not provide all of the features of claim 1. Nor does Shen *et al.* provide the missing

teaching as Shen *et al.* is concerned with using an oligonucleotide identification tag to assay reporter ligands in a sample. Shen *et al.* makes not mention of lipid bilayers, anchoring of biomolecules, or vesicles. Therefore, a prima facie case of obviousness of claim 8 has not been established.

The Commissioner is hereby authorized and requested to charge any deficiency in fees herein to Deposit Account No. 50-4616.

In view of the foregoing remarks, Appellants submit that the pending claims are in condition for allowance and patentably define over the prior art, and urge the Board to overturn the Examiner's rejections.

Respectfully submitted,
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Date: Aug. 5, 2011

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CLAIMS APPENDIX

1. (previously presented) An array of separated lipid bilayers, comprising

a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions,

a plurality of discrete lipid bilayer expanses in associated surface regions, said expanses having inner and outer bilayer surfaces,

an aqueous film interposed between each bilayer-compatible surface region and the lower surface of the corresponding lipid bilayer expanse,

each of said expanses containing one or more lipids derivatized with an oligonucleotide having a surface region specific oligonucleotide sequence and extending from the outer surface of the associated expanse,

a bulk aqueous phase covering the lipid bilayer expanses, and

at least one biomolecule anchored to at least one of the lipid bilayer expanses through a complementary oligonucleotide sequence capable of specifically hybridizing with the surface region specific oligonucleotide sequence in that expanse, such that the biomolecule is anchored to that expanse, wherein one or more of the biomolecules is a vesicle including at least one receptor associated with the vesicle, the receptor having a binding site located on the exterior of the vesicle and being capable of specifically binding a test agent.

2. (previously presented) The array of claim 1, wherein the array further includes one or more discrete lipid bilayer patches associated with said expanses, where each such patch contains a biomolecule anchored to the associated expanse through said hybridized oligonucleotides.

3. (previously presented) The array of claim 2, wherein the lipid bilayer expanses on different associated surface regions have different compositions.

4. (previously presented) The array of claim 3, wherein the oligonucleotide associated with each lipid bilayer expanse includes a different oligonucleotide.

6. (previously presented) The array of claim 2, further comprising one or more second biomolecules associated with at least one of the bilayer expanses, said second biomolecule(s) being able to move substantially freely within the associated expanse.

7. (previously presented) The array of claim 6, wherein at least some of the different bilayer expanses have different second biomolecules.

8. (previously presented) The array of claim 1, wherein the biomolecule is coupled to an oligonucleotide with a known sequence, such that the identity of the biomolecule may be determined from the sequence of the oligonucleotide.

9. (original) The array of claim 1, wherein said discrete lipid bilayer expanses in associated surface regions are separated by one or more barrier regions.

10. (original) The array of claim 1, wherein said discrete lipid bilayer expanses in associated surface regions are separated from one another by self-limiting lateral diffusion, without physical barriers between the expanses on the substrate surface.

11. (original) The array of claim 1, wherein said distinct bilayer-compatible surface regions on the substrate are formed from a material selected from the group consisting of SiO_2, MgF_2, CaF_2, and mica.

12. (previously presented) The array of claim 1, wherein the lipid bilayer expanses are comprised of phosphatidylcholine.

13. (withdrawn) A method of using the lipid patch array of claim 6 to detect membrane-bound biomolecular interactions, comprising

incubating the array under conditions effective to allow for the formation of biomolecular complexes between the second biomolecules, and

detecting any formed biomolecule complexes.

14. (withdrawn) The method of claim 13 for screening for molecules that enhance or disrupt membrane-bound biomolecular interactions, further comprising

contacting the array, prior to or after said incubating, with one or more molecules under conditions which allow for the interaction of said molecules with said biomolecules or biomolecular complexes,

detecting any formed biomolecular complexes, and

comparing the results from the previous step to the results from the detecting step of claim 13 to determine whether the one or more molecules enhanced or disrupted membrane-bound biomolecular interactions.

15. (withdrawn) The method of claim 13, wherein the degree of complex formation is quantitated.

16. (withdrawn) The method of claim 13, wherein said biomolecules are selected from the group consisting of peptides, proteins, carbohydrates, cytokines, growth factors, hormones, enzymes, toxins, drugs, oligonucleotides, lipids, and combinations thereof.

17. (withdrawn) The method of claim 13, wherein said molecules are selected from the group consisting of peptides, proteins, carbohydrates, cytokines, growth factors, hormones, enzymes, toxins, drugs, oligonucleotides, lipids, and combinations thereof.

18. (withdrawn) A method of manipulating lipid-bilayer regions on a substrate, comprising

applying, to the array of claim 1, a controlled laminar-flow stream of an aqueous liquid, under flow conditions effective to remove a portion of the expanse in the path of said stream, wherein remaining portions of said expanse are substantially retained in their original position(s) on said region, adjacent exposed portion(s) of said region.

21. (withdrawn) The array of claim 1, wherein the lipid bilayer expanses are comprised of at least one lipid selected from the group consisting of phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, and sphingomyelin.

22. (previously presented) The array of claim 1, wherein said receptor is a membrane protein.

23. (previously presented) The array of claim 22, wherein said membrane protein is a transmembrane protein.

24. (previously presented) The array of claim 1, wherein said vesicle has a size from between 30 to 200 nm.

25. (previously presented) The array of claim 1, wherein said oligonucleotide and complementary oligonucleotide have a length of between 16 to 24 nucleotides.

EVIDENCE APPENDIX

None

RELATED PROCEEDINGS APPENDIX

None